Evaluation of diagnostic coelioscopy in koi (*Cyprinus carpio*)

Brittany N. Stevens DVM

David Sanchez-Migallon Guzman LV, MS

Kathryn L. Phillips DVM

Alvin C. Camus DVM, PhD

Esteban Soto DVM, PhD

Received June 21, 2018.
Accepted August 7, 2018.

From the Departments of Veterinary Medicine and Epidemiology (Stevens, Sanchez-Migallon Guzman, Soto) and Veterinary Surgical and Radiological Sciences (Phillips), School of Veterinary Medicine, University of California-Davis, Davis, CA 95616; and the Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Camus). Dr. Stevens' present address is Aquarium of the Pacific, 100 Aquarium Way, Long Beach, CA 90802.

Address correspondence to Dr. Soto (sotomartinez@ucdavis.edu).

OBJECTIVE

To describe a technique for endoscopic evaluation of the coelomic viscera of koi (*Cyprinus carpio*) and to evaluate the ability to visually examine coelomic structures by use of an approach cranial or caudal to the pelvic girdle.

ANIMALS

16 subadult koi.

PROCEDURES

Koi were anesthetized with buffered tricaine methanesulfonate. Coelioscopic examination was performed via a ventral midline incisional approach cranial or caudal to the pelvic girdle. A 2.7-mm X 18-cm 30° oblique endoscope within a 4.8-mm operating sheath and infusion of saline (0.9% NaCl) solution was used. Ease of entry into the coelomic cavity and visual examination of structures were scored for each fish. Fish were euthanized 2 or 8 weeks after the procedure, and necropsy was performed.

RESULTS

The coelioscopic procedure was tolerated well, and all koi recovered uneventfully. For all fish, ease of entry and visual examination scores of the liver, intestines, gonads, heart, and anterior kidney were satisfactory to excellent. Visual examination of the posterior kidney and swim bladder was satisfactory to difficult, whereas the spleen and gallbladder were not visually identified. No significant differences were noted in entry or visual examination scores between the cranial and caudal approaches or between sexes. Minor complications included mild hemorrhage, rupture of the gonadal capsule, formation of adhesions between the viscera and incision site, and delayed healing of the incision.

CONCLUSIONS AND CLINICAL RELEVANCE

Diagnostic coelioscopy of koi appeared to be safe and effective. This procedure could have potential for use in examination of coelomic structures and disease diagnosis. (*Am J Vet Res* 2019;80:221–229)

Koi (*Cyprinus carpio*) are a domesticated variety of common carp and have gained immense popularity as ornamental fish because of their hardiness in captivity, longevity, and striking color patterns. It is becoming increasingly common for owners of ornamental fish to seek out veterinary care for their animals, and koi represent one of the most common fish species examined by exotic animal medicine clinicians. Clinical manifestations and diseases of the coelomic cavity and viscera such as ascites, egg binding, and neoplasia and swim bladder, hepatic, and renal diseases are frequently encountered in koi patients, and evaluation of coelomic distension is commonplace.

Coelioscopy (surgical laparoscopy) is a diagnostic tool that has been used in human and veterinary medicine for many years to evaluate internal structures. Coelioscopy allows a clinician to directly visually examine internal structures with the added benefit of providing magnification, and it can be performed in lieu of more invasive surgical techniques to collect biopsy specimens or to perform minimally invasive endosurgeries. The use of coelioscopy in exotic companion animal medicine has continued to steadily increase over the past few decades, and the use of coelioscopy to diagnose and treat a myriad of conditions in avian and reptilian patients has become routine.[1-3] However, despite the increasing use of coelioscopy in avian and reptilian patients, its use for the diagnosis and treatment of coelomic disease in ornamental teleosts has remained limited.

The use of coelioscopy in teleost fish dates back to 1984,[4] and coelioscopy has been used sporadically within the aquaculture and fisheries industries to determine fish sex, directly evaluate the coelomic viscera, or obtain gonad or liver biopsy specimens in a few teleost species.[5-14] The use of coelioscopy specifically in koi has been described in a few reports.[5,15,16] However, practitioners are often warned against performing coelioscopy in koi because of the fact that coelomic structures in koi typically are adhered by thin strands of connective tissue that may make coelioscopic examination more difficult, compared with performing the procedure in other species.[5,16] Despite this challenge of adherent con-

nective tissue, the high caseload of koi for exotic animal medicine practitioners and the high frequency of coelomic disease in koi patients make development of a safe and effective technique for performing coelioscopy in koi extremely desirable.

In most published reports,[6,7,9,10,12–14] the described entry site for the endoscope during coelioscopy of fish is located on the ventral midline and cranial to the pelvic girdle. However, most investigators were interested in targeting a specific tissue such as the gonads[6,7,10,13,14] or the cranially located liver.[9,12] Furthermore, most published reports describing coelioscopy in teleosts have been performed on species (eg, sturgeon[6,7,10,12,13] or channel catfish[9]) with a pelvic girdle located toward the caudal aspect of the ventrum in close proximity to the vent. The pelvic girdle of koi is amply located away from the vent in a more cranial position; therefore, endoscope entry sites could feasibly be cranial or caudal to the pelvic girdle.

The objectives of the study reported here were to describe a technique for the endoscopic evaluation of the coelomic viscera of koi and to evaluate the ability to visually examine coelomic structures by use of an approach cranial or caudal to the pelvic girdle. We hypothesized that coelioscopy in koi would be a safe procedure and provide satisfactory visual examination of all coelomic structures. In addition, we hypothesized that an endoscope entry site caudal to the pelvic girdle would allow for better visual examination of the caudal portions of the coelomic cavity while still providing satisfactory access to the cranial coelomic structures.

Materials and Methods

Animals

Sixteen juvenile (approx 4 months old) koi were obtained from a local koi producer.[a] The koi were transferred to the University of California-Davis Center for Aquatic Biology and Aquaculture and maintained in an outdoor, covered, 1.8-m-diameter, 1,556-L flow-through tank along with 30 similarly aged koi that were part of another study. Koi were maintained in this tank for approximately 14 months until the study was conducted. The study was approved by the University of California-Davis Institutional Animal Care and Use Committee (No. 19106).

The tank was supplied with fresh well water that was passed through a packed column aerator to allow removal of excessive nitrogenous products and ensure adequate oxygenation before being pumped to the animal systems. Flow rate of the tank was maintained at approximately 25 L/min, which allowed the entire volume of the tank to be exchanged approximately every hour. Tank temperature was monitored daily, dissolved oxygen content was monitored weekly, and concentrations of nitrogenous waste products were monitored monthly. Ambient water temperature ranged from 17° to 21°C throughout the 14-month maintenance period and from 17.5° to 19°C during the study period. Fish were fed a mixture of sinking[b] and floating[c] commercially pelleted fish food at a rate of 2.5% of body weight/d.

Anesthesia

Koi were approximately 18 months old at the time of the study. Food was withheld for 24 hours before anesthesia. On the day of the experiment, koi were transferred to the University of California-Davis Veterinary Medical Teaching Hospital in a 78-L container filled with maintenance tank water and supplied with air stones for aeration.

Koi were anesthetized by use of tricaine methanesulfonate[d] (also known as MS-222; 100 mg/L) buffered with sodium bicarbonate (100 mg/L) in an induction container filled with 10 L of maintenance tank water. Immediately after anesthesia was induced, a complete physical examination that included measurement of body weight, collection of morphometric measurements, collection of skin scrapings, and a gill biopsy was performed on each koi. Fish also underwent a complete ultrasonographic examination of the coelomic cavity as part of another unpublished study. After the ultrasonographic examination was completed, fish were administered hydromorphone[e] (0.7 mg/kg, IM) as a preoperative analgesic. Fish were then transferred to a recirculating anesthetic machine containing 10 L of maintenance tank water with buffered tricaine methanesulfonate (100 mg/L) for maintenance of anesthesia. Both the anesthetic induction chamber and recirculating anesthetic maintenance chamber were supplied with air stones to ensure adequate oxygenation of the water during the procedure.

Fish were placed in dorsal recumbency in a V-shaped trough positioned above the anesthetic chamber. A water pump was used to deliver a constant stream of anesthetic water across the gills of each fish by use of tubing placed in the fish's mouth **(Figure 1)**. Depth of anesthesia was monitored via the respiratory rate and response to a tail pinch. If the respiratory rate of a fish slowed substantially or respiration ceased for > 1 minute, untreated fresh water was used to bathe the gills and induce a lighter plane of anesthesia.

Coelioscopy

Skin on the ventral midline was prepared by spraying approximately 10 mL of sterile saline (0.9% NaCl) solution forcefully from a syringe onto the area of the intended incision, which was followed by gentle wiping of the area with a sterile gauze square. Sterile hemostats were then used to remove 1 or 2 scales in the area immediately overlying the area of the intended incision. Fish were randomly allocated by use of a computer software program[f] into 2 groups (8 fish/group). A No. 15 scalpel blade was used to make a skin incision (5 to 7 mm in length) and partial incision of the musculature 1 cm cranial to the pelvic girdle in one group and 1 cm caudal to the pelvic girdle in the other group (Figure 1). Edges of the incision were then grasped with forceps and elevated, and the

Figure 1—Photographs of koi (*Cyprinus carpio*) positioned for anesthesia (A), the intended incisional sites for coelioscopy (B), and insertion of the coelioscope via an approach cranial to the pelvic girdle (C). A—A koi is positioned in a V-shaped plexiglass platform lined with a synthetic nonabrasive chamois located on top of a small 10-L tank, and a small submersible pump is used to pump water through a rubber tube to maintain anesthesia. B—Location of incisions on the ventral aspect of a koi for a coelioscopic approach that is cranial (a) or caudal (b) to the pelvic girdle. C—Insertion of the coelioscope in a koi by use of the cranial approach. Notice the location of the incision in relationship to the pelvic fins.

coelomic cavity was entered by use of an inverted No. 15 scalpel blade. A 2.7-mm X 18-cm 30° oblique endoscope[g] housed within a 4.8-mm operating sheath[g] that was connected to an endovideo camera,[g] monitor, and halogen light source attached to a fiber-optic cable[g] was then introduced into the coelomic cavity. A 1-L bag of saline solution was connected to one of the ports of the operating sheath via a sterile IV fluid line, and saline solution was infused to create working space to allow visual examination of the viscera. Endoscopes, sheaths, and surgical instruments were cold sterilized by immersion in 2.4% glutaraldehyde (20-minute soak-rinse cycle) prior to each procedure.

Time to coelomic entry was defined as the interval from incision to the time that the structures could first be visually identified. Ease of entry was scored on a scale of 1 to 5 by use of previously developed criteria[17] (1 = impossible [requires > 15 minutes], 2 = difficult [requires between 11 and 15 minutes], 3 = satisfactory [requires between 6 and 10 minutes], 4 = good [requires between 2 and 5 minutes], and 5 = excellent [requires < 2 minutes]). Ease of location and visual examination of the posterior kidney, anterior kidney, gonads, intestines, swim bladder, liver, gallbladder, spleen, and heart were scored on a scale of 1 to 5 by use of another previously published scale[17] (1 = impossible, 2 = difficult [requires an extensive search and substantial manipulation of viscera], 3 = satisfactory [requires some searching and minor manipulation of viscera], 4 = good [easy to locate but requires minor manipulation to clearly see the structure], and 5 = excellent [obvious and clear view with minimal or no manipulation required]). Sex of each koi was determined by direct visual examination of the gonads.

After the coelioscopic examination was completed, the endoscope and operating sheath were removed, and remaining excess saline solution was manually expressed from the coelomic cavity. The incision was closed with 3-0 poliglecaprone 25[h] in a full-thickness simple interrupted or cruciate pattern. Koi then received meloxicam[i] (0.5 mg/kg, IM) and ceftiofur crystalline-free acid[j] (20 mg/kg, IM). Fish were moved to a 78-L freshwater recovery container and monitored until their equilibrium returned and they were swimming normally. After the koi recovered from anesthesia, they were returned to their original source tank and monitored daily.

Necropsy and histologic evaluation

Koi were euthanized by administration of an overdose of buffered tricaine methanesulfonate (≥ 500 mg/L) at 2 or 8 weeks after coelioscopy (8 fish/time point). Necropsy was performed on all fish. Particular attention was given to any evidence of internal trauma. The skin incision site and any abnormal-appearing internal tissues were collected and placed in neutral-buffered 10% formalin for fixation. Tissues were processed routinely, embedded in paraffin, sectioned at a thickness of 5 µm, stained with H&E stain, and examined microscopically by personnel at the Department of Pathology, College of Veterinary Medicine, University of Georgia.

Statistical analysis

A nonparametric procedure[k] was used to compare approaches (cranial and caudal) and sex (male and female) by use of the Wilcoxon rank sum test with regard to scores for ease of entry or visual examination scores of structures. Score was then considered as a pseudometric variable, and a general linear model procedure was used to analyze the data as a 2 X 2 factorial with approach and sex as the 2 factors. All comparisons were considered significant at $P \leq 0.05$.

Results

Mean ± SD body weight of the koi was 384 ± 168 g, and mean total length was 28.2 ± 3.5 cm. Most fish appeared to be healthy and in good body condition, although a few fish had minor skin and fin

Table 1—Visual examination scores for 16 koi (*Cyprinus carpio*) undergoing coelioscopy on the basis of approach to the pelvic girdle and sex of fish.

	Cranial approach (n = 8)		Caudal approach (n = 8)		Male (n = 9)		Female (n = 7)	
Structure	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Posterior kidney	3	1–3	3	1–3	3	1–3	3	1–3
Anterior kidney	4	1–5	4	3–4	4	3–4	4	1–4
Gonads	5	4–5	5	5–5	5	5–5	5	4–5
Intestines	5	2–5	5	4–5	5	3–5	5	4–5
Swim bladder	3	2–4	2.5	2–3	2	2–3	3	1–3
Liver	5	5–5	5	5–5	5	5–5	5	5–5
Gallbladder	1	1–1	1	1–1	1	1–1	1	1–1
Spleen	1	1–1	1	1–1	1	1–1	1	1–1
Heart and pericardium	4	4–5	4	4–4	4	4–4	4	4–4

Visual examination scores were defined as follows: 1 = impossible, 2 = difficult (requires an extensive search and substantial manipulation of viscera), 3 = satisfactory (requires some searching and minor manipulation of viscera), 4 = good (easy to locate but requires minor manipulation to clearly see the structure), and 5 = excellent (obvious and clear view with minimal or no manipulation required).

wounds or proliferative skin lesions that appeared grossly consistent with carp pox (a condition caused by infection with cyprinid herpesvirus 1).

All fish were anesthetized without complication. The sex of each fish (9 males and 7 females) was readily and accurately identified via coelioscopy, although 1 fish had only 1 testis present on the right side of the body. This finding was later confirmed via necropsy. Ease of entry scores were excellent for all fish, regardless of the approach used (median value, 5 [range, 4 to 5] for both the cranial and caudal approaches) or sex of the fish (median value, 5 [range, 4 to 5] for both males and females).

Visual examination scores were summarized **(Table 1)**. Endoscopic location and visual examination scores were good to excellent (median, ≥ 4) for the anterior kidney, gonads, intestines, and liver **(Figure 2)**. Visual examination scores for the posterior kidney (median, 2.5) and swim bladder (median, 3) were satisfactory to difficult, whereas the spleen and gallbladder were not visually identified during the procedure (median, 1). The spleen is located medial to the intestinal loops, mesenteric fat, left lobe of the liver, and pancreatic tissue in the mid coelom of koi, whereas the gallbladder is similarly located medially on the cranial aspect of the right side of the body, where it is surrounded by the right lobe of the liver. In the first koi, the liver lobes as well as the mesenteric fat were friable when manipulated with the endoscope; thus, after a few initial attempts to locate these structures, it was deemed ill-advised to continue to probe between the loops of intestines and liver to visually examine these structures, and it was not attempted in subsequent fish.

Ease of entry scores or visual examination scores for any structure did not differ significantly between the cranial versus caudal approach or between males and females. Minor complications during coelioscopy included minor bleeding that momentarily obscured the field of vision in 2 fish and rupture of the capsule of the gonad in 6 fish, which resulted in the release of a small number of eggs or amount of milt into the coelomic cavity in 3 of the 6 fish. All fish recovered from the procedure without additional complications and resumed eating by the following day. No behavioral abnormalities were noted during the 2- or 8-week postoperative monitoring period.

Necropsy of the 8 koi 2 weeks after the procedure revealed incomplete healing as well as moderate to severe swelling and redness of the skin at the endoscope insertion site **(Figure 3)**. Mild to moderate adhesion of the underlying viscera to the incision site was apparent in 2 of the 8 fish. Additionally, a mild hematoma at the incision site was noted in 2 of the 8 fish. In 1 female, iatrogenic rupture of the ovarian capsule was evident near the posterior kidney, and there was an area (approx 1 X 2 cm) of extruded eggs. In the other 3 fish in which there was rupture of the gonad capsule during the procedure, there was no gross evidence of capsular rupture observed during necropsy or evidence of abnormal adhesions of the gonads to other structures or the peritoneal wall. There was no gross evidence of inflammation or damage to the peritoneal wall or serosal surfaces of the structures caused by insufflation of saline solution during the procedure.

Histologic examination of the insertion sites 2 weeks after coelioscopy revealed good apposition of surgical margins in all samples, and reepithelialization was complete or nearly complete in 5 of the 8 fish. However, reepithelialization and the intensity of inflammatory and fibroblastic responses differed markedly among fish and among various areas of the same incision **(Figure 4)**. Bridging of incision margins by fibroblasts was minimally evident in only 1 fish. Incision margins, particularly around sutures, were bordered by epithelial hyperplasia accompanied by diffuse, mild intracellular edema and infiltration by small numbers of neutrophils and lymphocytes. Congestion and hypertrophy of capillary endothelial cells was mild to moderate in the superficial dermis. Scales adjacent to the incision were un-

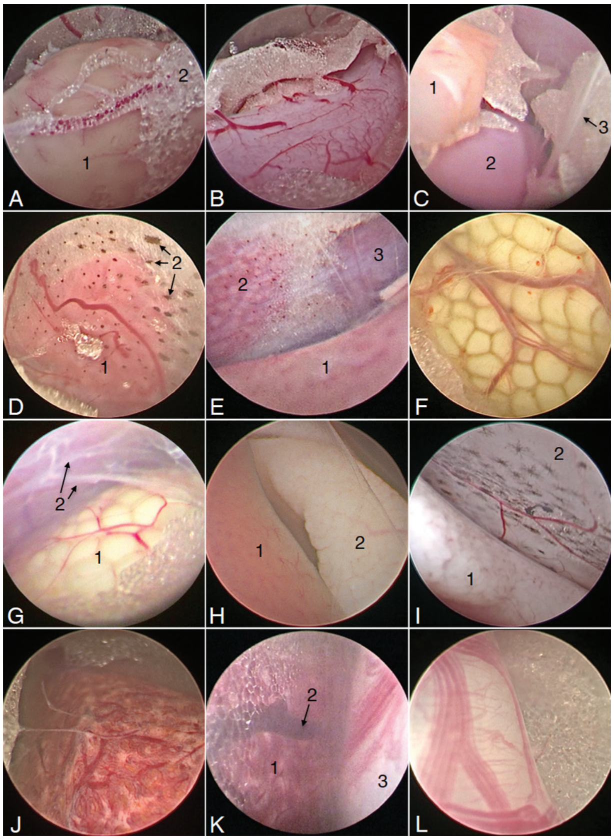

Figure 2—Endoscopic images obtained during coelioscopy of a koi. A—Liver (1) and coelomic fat (2). B—Intestine. C—Liver (1), intestine (2), and fibrous connective tissue attaching viscera to the peritoneal wall (3). D—Anterior kidney covered by the coelomic membrane (1) and chromatophores on the coelomic membrane (2). E—Cranial aspect of the coelom (dorsal is toward the bottom of the image) revealing the liver (1), anterior kidney (2), and heart covered by the pericardial membrane (3). Cardiac contractions make the heart readily distinguishable during coelioscopy. F—Ovary with ova visible through the transparent capsule or tunica albuginea of the ovary. G—Ovary (1) and fibrous connective tissue attaching the ovary to the peritoneal wall (2). H—Liver (1) and testis (2). I—Testis (1) and peritoneal wall (2). J—Posterior kidney. Notice that the posterior kidney is not retrocoelomic, which is in contrast to the anterior kidney. K—Posterior kidney (1) with a large renal blood vessel (2) and the swim bladder (3). L—Swim bladder with characteristic pattern of large blood vessels.

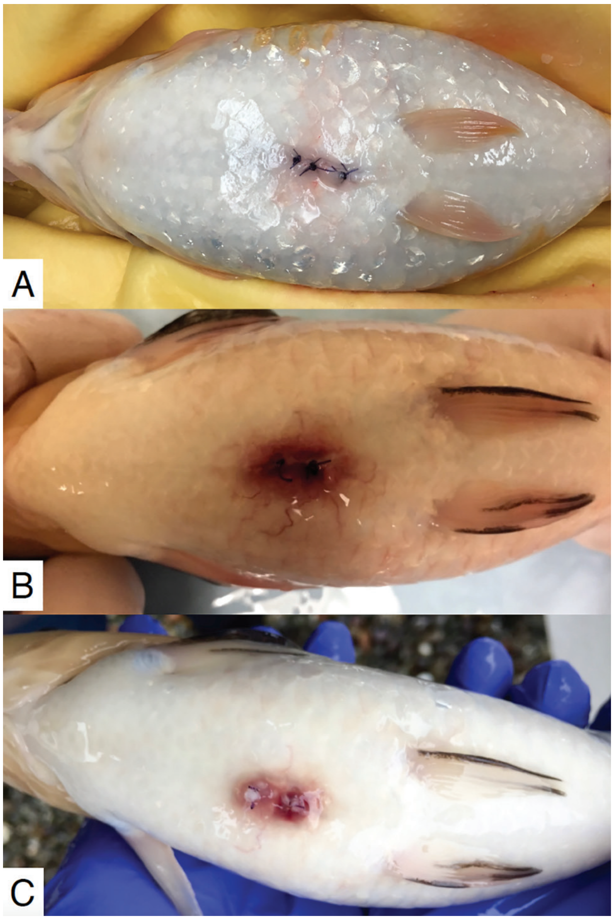

Figure 3—Photographs of the incision site on the ventral aspect of koi immediately (A), 2 weeks (B), and 8 weeks (C) after coelioscopy. Notice the redness of the skin at the endoscope insertion site at weeks 2 and 8.

dergoing osteoclastic resorption in 2 of the 8 fish. Incision defects were filled by minimal to large fibrin clots containing mixtures of RBCs, neutrophils, macrophages, and necrotic cellular debris. Margins of the clots were beginning to be infiltrated by plump proliferating fibroblasts that dissipated within the adjacent dermis, hypodermis, and skeletal muscle. Similarly, associated inflammatory changes, which were dominated by neutrophils, with smaller numbers of macrophages and scattered lymphocytes radiated multifocally from incision sites. Changes were severe in 1 fish but mild to moderate in the other 7 fish. Inflammatory infiltrates dissected endomysial areas and isolated individual and variably sized groups of myofibers, often in association with suture material. There was prominent regeneration of muscle, characterized by disparity in myofiber diameter, an increased amount of basophilia, nuclear hypertrophy and centralization, and satellite cell proliferation in 7 of the 8 fish. Mild necrosis and saponification of fat with mild hemorrhage, edema, a mixed inflammatory reaction, and fibroplasia were scattered throughout the hypodermis. Additional inconsistent findings included fragments of sequestered bone within the dermis and hypodermis. Organizing fibroblastic adhesions between liver lobes and the peritoneum were evident in 3 fish, and severe perirenal fibroplasia was evident in 1 fish.

Necropsy of the 8 koi 8 weeks after the procedure also revealed incomplete healing as well as moderate to severe swelling and redness of the skin at the endoscope insertion site, although swelling and redness of the skin associated with the incision site were subjectively less severe than in the fish necropsied 2 weeks after the procedure (Figure 3). Microscopically, the incisions of 2 of the 8 fish were characterized by nearly complete wound healing **(Figure 5)**. Incision surfaces were covered by a continuous, well-ordered epithelium of normal height that contained goblet cells, large pale alarm or club cells, and scat-

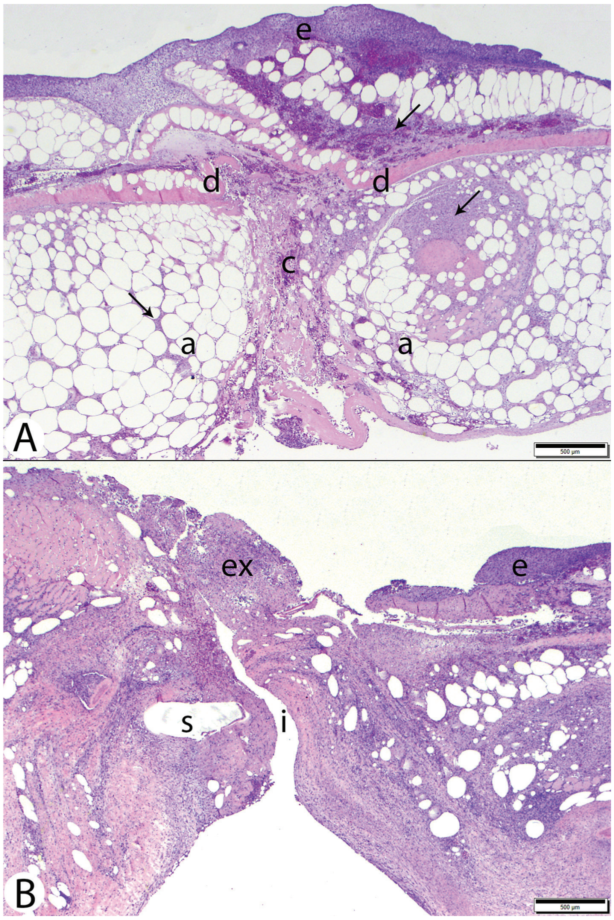

Figure 4—Photomicrographs of skin samples obtained from the incision site of koi 2 weeks after coelioscopy. A—The image depicts delayed healing. The epithelium (e) is continuous, but incision margins are separated by a large fibrinohemorrhagic clot (c) throughout the body wall, which includes the dermis (d). Mild to moderate pyogranulomatous inflammatory infiltrates (arrows) are scattered throughout the superficial dermis and subcutaneous adipose tissue (a). B—The image depicts poor healing. The epithelium (e) is incomplete, and the wound surface is partially covered by a layer of serofibrinous crust and cellular debris (ex). Sides of the incision (i) have separated, and severe pyogranulomatous inflammation extends transmurally through the section, which severely disrupts the normal tissue architecture. Suture material (s) is visible. H&E stain; bar = 500 µm.

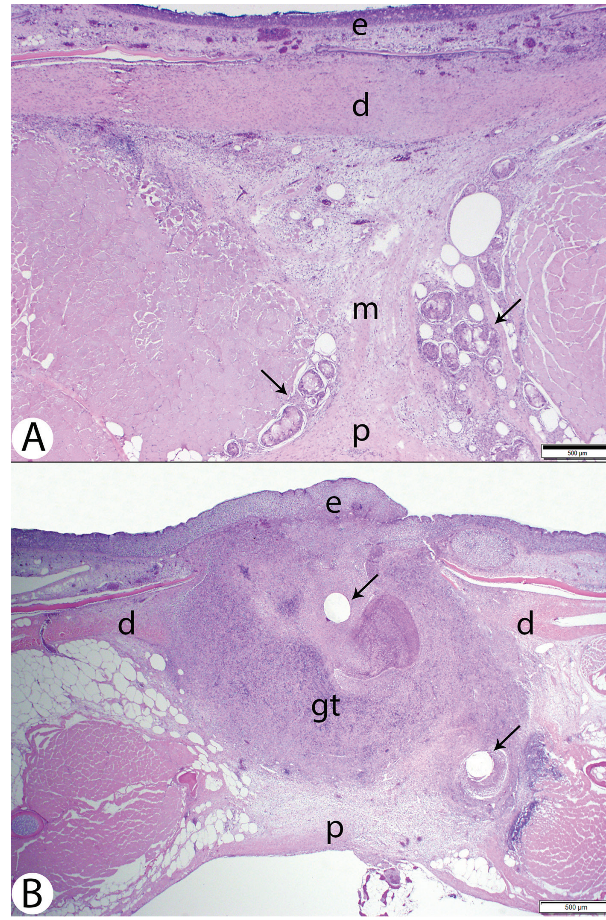

Figure 5—Photomicrographs of skin samples obtained from the incision site of koi 8 weeks after coelioscopy. A—The image depicts good healing. There is a continuous epithelium (e) that has normal height. The dermis (d) and peritoneal wall (p) are continuous and composed of organized collagen bundles. The hypodermal layer and muscle layer (m) are contracted with minimal residual inflammation. Granulomas containing necrotic fat (arrows) are evident. B—The image depicts poor healing. The epithelium (e) is continuous but hyperplastic and edematous. However, the dermis (d) and peritoneal wall (p) are not continuous. The dermis, hypodermal layer, and muscle layer contain a large area of granulomatous inflammation (gt) and focal suppuration surrounding suture sites (arrows). H&E stain; bar = 500 µm.

tered lymphocytes. Small numbers of lymphocytes and neutrophils were present in the superficial dermis immediately adjacent to the incision. The compact dermis was contiguous and dominated by well-organized parallel collagen bundles, with only a few scattered fibroblasts. Regenerating scales were lined by plump osteoblasts. In the underlying muscle and adipose tissues, the incisional defect was filled by a more disorganized mixture of areolar connective tissue and scattered fibroblasts mixed with small numbers of regenerating myofibers. Deep and peripheral to this organizing fibrous scar, the incision area was bordered by a narrow zone of inflammatory cells dominated by macrophages with fewer lymphocytes and scattered neutrophils that enveloped multifocal granulomas. The granulomas had central regions of wispy pale eosinophilic material (which were interpreted as necrotic fat) surrounded by mantles of epithelioid cells and multinucleated giant cells. The peritoneal wall was continuous and similar in appearance to the compact dermis.

In the remaining 6 fish, the epithelium had variable degrees of hyperplasia and intracellular edema with infiltration by small numbers of lymphocytes. There was no continuity of the compact dermis. Extensive mixtures of granulation tissue, sheets of granulomatous inflammation, and foci of suppurative inflammation expanded the dermis and extended to variable depths in the hypodermal and muscle layers. Inflammatory changes were most severe in association with the presence of persistent suture material.

Healing in the deeper portions of the body wall was similar to that previously described for the first 2 fish in the group, including granulomas. Mild to moderate adhesion of the underlying viscera to the incision site was grossly apparent in 6 of the 8 fish. In the 2 fish in which there was rupture of the gonad capsule during coelioscopy, there was no gross evidence of capsular rupture or evidence of abnormal adhesions of the gonads to other structures or the peritoneal wall. Microscopically, continuity of the peritoneal wall was complete to nearly complete in all fish. Seven fish had variably sized adhesions of the incision site to the liver, mesenteric adipose tissue and pancreas, or ovaries. In 1 fish, liver tissue was trapped within the contracting incision margins.

Discussion

The coelioscopic technique for the study described here was based on recommendations and guidelines for examination of teleosts[4–14] and proved to be a safe and effective method for the evaluation of the coelomic viscera of koi. The equipment used in the present study typically can be found in clinics of exotic animal medicine practitioners and can be readily used for examination of teleost patients. Premedication with hydromorphone, induction with buffered tricaine methanesulfonate (100 mg/L), and maintenance with buffered tricaine methanesulfonate (100 mg/L) by use of a recirculating anesthetic system proved to be an effective anesthetic regimen for the procedure. The use of saline solution for insufflation allowed for sufficient distension of the coelomic cavity and excellent visibility of structures, and it did not result in buoyancy disorders after coelioscopy, as may have been seen had compressed gas been used. There were excellent visual examination scores for the liver, intestines, and gonads, and these structures were readily visible immediately on entry of the endoscope into the ventral aspect of the coelomic cavity. Advancing the endoscope cranially along the ventral midline or the lateral body wall also enabled us to locate the more cranial structures of the heart and anterior kidney with relative ease. Visual examination of the dorsally located posterior kidney and swim bladder required more manipulation and searching to achieve a satisfactory view. To view these structures, it is recommended that the endoscope be advanced dorsally along the lateral body wall while pushing the viscera medially. In our experience, it is possible for an experienced operator to perform an endoscopic evaluation of the coelomic cavity of koi in < 10 minutes.

In the study reported here, visual examination scores did not differ significantly between the cranial or caudal approach. We speculate that this lack of difference in visual examination scores for the dorsally and caudally located structures was attributable to the fact that regardless of the approach used, the amount of searching (moving from a ventral insertion site to the dorsal aspect of the coelomic cavity) and viscera manipulation (pushing the viscera to the side) that was required to visually examine these structures was comparable. However, it should be mentioned that although the caudally located structures could be viewed from the cranial approach, the angle required to view these structures by use of the cranial approach was much more cumbersome, compared with that for the caudal approach. For example, the saddle-shaped portion of the posterior kidney that is located between the lobes of the swim bladder was situated almost perpendicular to an incision made just caudal to the pelvic girdle, whereas there was an angle of approximately 120° when this structure was approached by use of an incision made cranial to the pelvic girdle. Thus, despite the lack of significant differences in visual examination scores between the 2 approaches, we recommend the use of an approach caudal to the pelvic girdle for the ease and comfort of the endoscopist when attempting to view structures in the caudal portion of the coelomic cavity in fish of the size in the present study. Furthermore, we speculate that if ease of angle of approach were added as an additional criterion to the visual examination scoring system, a significant difference would be detected in the visual examination scores of the posterior kidney and swim bladder between the cranial and caudal approach.

In the present study, the anatomic location of the spleen and gallbladder made endoscopic evaluation of these structures challenging. Koi have multiple hepatic lobes and pancreatic tissue that are located along with mesenteric fat between loops of intestine. To view the spleen or gallbladder, which are located medially, it is necessary to probe between the intestinal loops. This proved to be difficult during coelioscopy because the hepatic and pancreatic tissue as well as the mesenteric fat were quite friable and had many small blood vessels that were easily disrupted. Therefore, we do not recommend that clinicians attempt to evaluate the spleen or gallbladder via coelioscopy because iatrogenic damage to the surrounding viscera may occur.

Another challenge that was encountered in the study reported here was the iatrogenic rupture of the thin gonadal capsule during coelioscopic manipulation. This was most commonly encountered when attempting to visually examine the posterior kidney and swim bladder because viewing these structures required that the gonads be moved medially. Rupture of the gonadal capsule occurred during coelioscopy in 6 of 16 fish. However, at the time of necropsy, gross evidence of capsular damage was noted in only 1 fish. Similar to findings for the present study, results of other studies in which the gonads were intentionally biopsied by use of coelioscopy indicated that minor trauma to the gonads was tolerated well by fish.[6,7,10,13] However, despite the apparent resilience of fish to gonadal capsular trauma, avoidance of iatrogenic trauma may be possible through careful and cautious

manipulation of the endoscope. It is further recommended that when clinically feasible, coelioscopy should not be performed during the spawning season because the gonads will be at their maximal size and friability during that time.

Koi have thin strands of adherent connective tissue that connect the visceral structures to each other and to the peritoneal wall. This unique attribute of koi anatomy has caused authors to discourage the use of coelioscopy in koi.[5,16] In the study reported here, thin strands of connective tissue were commonly encountered; however, we did not find the presence of these structures to be an insurmountable obstacle because most structures could still be visually examined with satisfactory to excellent success. However, care must be taken during insertion of an endoscope because the intestines and liver tissue are located immediately below the intended insertion site and are often attached to the peritoneal wall, which makes iatrogenic damage a potential risk. Formation of more adhesions between the viscera and between the viscera and body wall is likely in fish with coelomic diseases that lead to an increase in inflammation. Fish of the present study were healthy; therefore, they had only the amount of adherent connective tissue that would typically be encountered in koi. It may be that fish with active coelomic disease would have excessive adhesion formation that could preclude satisfactory coelioscopic evaluation. Furthermore, similar to results in other species (eg, trout[4]), repeated coelioscopy of koi may lead to an increase in adhesion formation. Practitioners should be aware of this potential sequela when multiple coelioscopic procedures are necessary. Evidence of an increase in adhesion formation after coelioscopy was detected in the koi of the present study during necropsy because there was more substantial adhesion formation between the intestines, hepatic tissue, and pancreatic tissue and the peritoneal wall in the area of the incision site in 8 of 16 fish.

A final complication for the koi of the present study was delayed incision healing (16/16 incisions were not completely healed at 2 weeks and 8/16 were not completely healed at 8 weeks after coelioscopy). Factors such as water temperature and type of suture material can affect the rate of wound healing in teleosts.[18–22] The study reported here was performed during the winter (January and February); thus, seasonally cooler water temperatures and subsequently lower metabolic rates of the fish likely played a role in the delayed healing.[18] The effect of suture material on wound healing has been evaluated in a number of studies of teleosts,[19–21] including koi.[22] A general consensus among the investigators of these studies is that absorbable monofilament suture is associated with the most satisfactory healing outcomes.[20–22] Subsequently, poliglecaprone 25 was chosen for use in the present study. Histologic examination of the incision sites revealed a severe inflammatory reaction associated with the suture material. Thus, poliglecaprone 25 may not be the ideal suture for koi, and improved wound healing may result if other absorbable monofilament sutures are chosen for closure. Removing sutures a few weeks after procedures, rather than letting them dissolve naturally, may also help limit suture-associated inflammation and has been recommended by some authors[23] on the basis that absorbable sutures hydrolyze much more slowly in ectotherms than in endotherms.

Despite some inherent challenges of koi anatomy, diagnostic coelioscopy of koi for the evaluation of the liver, intestines, heart, gonads, anterior and posterior kidneys, and swim bladder appeared to be safe, effective, and relatively easy to perform. If disease of one of these structures is suspected, we advocate use of the technique described here for visual examination of the coelomic cavity. On the basis of our results for the present study, we do not advocate the use of coelioscopy to investigate diseases of the gallbladder or spleen because of their anatomic location and concerns about iatrogenic damage to surrounding viscera. Although there was not a significant difference in ease of entry into the coelomic cavity or visual examination scores between the cranial and caudal approaches, we recommend for the ease and comfort of the endoscopist that an approach caudal to the pelvic girdle be used when dorsally and caudally located structures (eg, swim bladder or posterior kidney) are examined in koi.

Acknowledgments

Support for Dr. Stevens was provided by an Aquatic Animal Health fellowship from the California Academy of Science (Steinhart Aquarium), Monterey Bay Aquarium, Hubbs Sea World Research Institute, and University of California-Davis Veterinary Medical Teaching Hospital.

The authors thank Alexi Haack and Denver Coleman for assistance with this project and Anthony Quintero for providing the koi used in this study.

Footnotes

a. Koi Enterprise, West Sacramento, Calif.
b. Classic Fry, Skretting, Tooele, Utah.
c. Koi color, Skretting, Tooele, Utah.
d. Western Chemicals Inc, Ferndale, Wash.
e. Hospira Inc, Lake Forest, Ill.
f. QuickCalcs, Graphpad Software Inc, La Jolla, Calif.
g. Karl Storz Veterinary Endoscopy America Inc, Goleta, Calif.
h. Monocryl, Ethicon US LLC, Bridgewater, NJ.
i. Metacam, Boehringer-Ingelheim, St Joseph, Mo.
j. Excede for swine, 100 mg/mL, Pfizer Animal Health, New York, NY.
k. NPAR1WAY, SAS, version 9.4, SAS Institute Inc, Cary, NC.

References

1. Divers SJ. Endoscopy equipment and instrumentation for use in exotic animal medicine. *Vet Clin North Am Exot Anim Pract* 2010;13:171–185.
2. Divers SJ. Reptile diagnostic endoscopy and endosurgery. *Vet Clin North Am Exot Anim Pract* 2010;13:217–242.
3. Divers SJ. Avian diagnostic endoscopy. *Vet Clin North Am Exot Anim Pract* 2010;13:187–202.
4. Moccia RD, Wilkie EJ, Munkittrick KR, et al. The use of fine needle fibre endoscopy in fish for in vivo examination of vis-

ceral organs, with special reference to ovarian evaluation. *Aquaculture* 1984;40:255–259.

5. Murray MJ. Fish surgery. *Semin Avian Exotic Pet Med* 2002;11:246–257.
6. Hernandez-Divers SJ, Bakal RS, Hickson BH, et al. Endoscopic sex determination and gonadal manipulation in Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotoi*). *J Zoo Wildl Med* 2004;35:459–470.
7. Hurvitz A, Jackson K, Degani G, et al. Use of endoscopy for gender and ovarian stage determinations in Russian sturgeon (*Acipenser gueldenstaedtii*) grown in aquaculture. *Aquaculture* 2007;270:158–166.
8. Swenson EA, Rosenberger AE, Howell PJ. Validation of endoscopy for determination of maturity in small salmonids and sex of mature individuals. *Trans Am Fish Soc* 2007;136:994–998.
9. Boone SS, Hernandez-Divers SJ, Radlinsky MG, et al. Comparison between coelioscopy and coeliotomy for liver biopsy in channel catfish. *J Am Vet Med Assoc* 2008;233:960–967.
10. Divers SJ, Boone SS, Hoover JJ, et al. Field endoscopy for identifying gender, reproductive stage and gonadal anomalies in free-ranging sturgeon (*Scaphirhynchus*) from the lower Mississippi River. *J Appl Ichthyol* 2009;25(suppl 2):68–74.
11. Carreiro CR, Furtado-Neto MA, Mesquita PE, et al. Sex determination in the giant fish of Amazon Basin, *Arapaima gigas* (*Osteoglossiformes, Arapaimatidae*), using laparoscopy. *Acta Amazon* 2011;41:415–419.
12. Divers SJ, Boone SS, Berliner A, et al. Nonlethal acquisition of large liver samples from free-ranging river sturgeon (*Scaphirhynchus*) using single-entry endoscopic biopsy forceps. *J Wildl Dis* 2013;49:321–331.
13. Munhofen JL, Jiménez DA, Peterson DL, et al. Comparing ultrasonography and endoscopy for early gender identification of juvenile Siberian sturgeon. *N Am J Acquac* 2014;76:14–23.
14. Macrì F, Rapisarda G, De Stefano C, et al. Coelioscopic investigation in European eels (*Anguilla anguilla*). *J Exot Pet Med* 2014;23:147–151.
15. Saint-Erne N. Diagnostic techniques and treatments for internal disorders of koi (*Cyprinus carpio*). *Vet Clin North Am Exot Anim Pract* 2010;13:333–347.
16. Stetter MD. Diagnostic imaging and endoscopy. In: Wildgoose WH, British Small Animal Veterinary Association, eds. *BSAVA manual of ornamental fish*. Gloucester, England: British Small Animal Veterinary Association, 2001;103–108.
17. Divers SJ, Stahl SJ, Camus A. Evaluation of diagnostic coelioscopy including liver and kidney biopsy in freshwater turtles (*Trachemys scripta*). *J Zoo Wildl Med* 2010;41:677–687.
18. Anderson CD, Roberts RJ. A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. *J Fish Biol* 1975;7:173–182.
19. Deters KA, Brown RS, Carter KM, et al. Performance assessment of suture type, water temperature, and surgeon skill in juvenile Chinook salmon surgically implanted with acoustic transmitters. *Trans Am Fish Soc* 2010;139:888–899.
20. Thorstad EB, Økland F, Westerberg H, et al. Evaluation of surgical implantation of electronic tags in European eel and effects of different suture materials. *Mar Freshw Res* 2013;64:324–331.
21. Boone SS, Hernandez SM, Camus AC, et al. Evaluation of four suture materials for surgical incision closure in Siberian sturgeon. *Trans Am Fish Soc* 2013;142:649–659.
22. Hurty CA, Brazik DC, Law JM, et al. Evaluation of the tissue reactions in the skin and body wall of koi (*Cyprinus carpio*) to five suture materials. *Vet Rec* 2002;151:324–328.
23. Harms CA. Surgery in fish research: common procedures and postoperative care. *Lab Anim (NY)* 2005;34:28–34.